

REMARKS

With the current Response, claims 2 and 4 are canceled. Further, claims 1, 3 and 5 are amended. As such, claims 1, 3 and 5-12 are currently pending. The amendments find support in the specification and are discussed in the relevant sections below. No new matter is added.

Claims Rejected Under 35 U.S.C. §103(a):

The Office Action of December 29, 2004 rejected claims 1-12 under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent No. 6,007,690 to Nelson et al. ("the Nelson et al. patent") in view of a reference published in Rapid Communications in Mass Spectrometry by Wang et al. ("the Wang et al. reference").

More specifically, the December 29th Office Action stated:

The reference of Nelson et al. discloses a microfluidic device that includes an inlet channel (66); a reaction channel (enrichment channel, 62) and solid supports (3) in communication with the reaction channel and capable of concentrating a charged analyte produced by a reaction in the reaction channel.

While the reference of Nelson et al. discloses that the reaction channel (enrichment channel, 62) may be used as a microreactor for protein digestion (See column 4, lines 43-67), the reference does not specifically disclose that enzyme is located in the channel.

The reference of Wang et al. clearly discloses that it is conventional in the art to provide enzyme within a reaction channel on a microfluidic device (See Figure 1 and related disclosure).

In view of this teaching, it would have been obvious to one of ordinary skill in the art at the time the invention was made to provide the enrichment channel (62) of the reference of Nelson et al. with an enzyme for the known and expected result of providing an art recognized means for protein digestion so as to provide a microreactor as suggested by the reference of Nelson et al.

With respect to the specifics of the membrane employed of claims 2-4, the reference of Nelson et al. discloses a number of possible solid supports that can be employed with respect to the enrichment channel (See column 6, lines 1-56). As a result, in the absence of a showing of criticality and/or unexpected results, it would have been obvious to one of

ordinary skill in the art at the time the invention was made to determine the optimum material for enclosing the enrichment channel based merely on the specifics of the analyte to be reacted and/or detected in the system.

With respect to the charge on the membrane of claims 5-10, based merely on the specific material of the membrane employed, the material will inherently include a positive or negative charge. Additionally, it would have been obvious to one of ordinary skill in the art to provide a desired charge for the capture of reaction products as suggested by the reference of Nelson et al. (See column 6, lines 46-53).

With respect to the side channels of claims 6-10, the reference of Nelson et al. discloses the use of side channels (14, 15).

With respect to the electrodes of claims 9 and 10, the reference of Nelson et al. discloses the use of electrodes (60 and 61) with respect to the side channels. As is known in the field of electrophoresis, the voltage applied to the electrodes can be positive or negative based merely on the desired direction of flow. As a result, the electrodes of Nelson et al. are structurally capable of being positive or negative.

With respect to the claimed upstream module of claim 11, it would have been obvious to one of ordinary skill in the art to purify the sample prior to introduction into the microreactor system for the known and expected result of removing any components of the sample which may interfere with the analysis reactions and/or detection.

With respect to the downstream separation module of claim 12, the reference of Wang et al. discloses that it is conventional in the art to provide the protein digested sample of a microfluidic device to a MS for further separation and analysis (See Figure 1 and related text). As a result, it would have been obvious to one of ordinary skill in the art at the time the invention was made to further analyze the reaction products of the device of Nelson et al. using a MS as suggested by the reference of Wang et al. (December 29, 2005 Office Action; Pages 3-5).

With this Response, Applicant has amended independent claim 1 to require, a microfluidic device, comprising: an inlet channel; an at least one reaction channel engaged to the inlet channel wherein an enzyme is located within the reaction channel; and **an at least one membrane having a plurality of pores** in communication with the reaction channel capable of concentrating a charged analyte produced by a reaction in the reaction channel, **wherein the pores of the membrane have a pore diameter greater than a diameter of the charged analyte**. The cited art does not disclose, teach or suggest a microfluidic device capable of

concentrating a charged analyte with a membrane wherein the pores of the membrane are larger than the diameter of the analyte. As such, Applicant respectfully requests reconsideration and allowance of pending claims 1, 3 and 5-12.

As amended, independent claim 1 requires a microfluidic device comprising **an at least one membrane having a plurality of pores** in communication with the reaction channel capable of concentrating a charged analyte produced by a reaction in the reaction channel, **wherein the pores of the membrane have a pore diameter greater than a diameter of the charged analyte**. As disclosed and taught in the specification as filed, it is possible to retain these small diameter analytes with a membrane having a large pore diameter (the pore diameter being at least larger than the diameter of the analytes) because the analytes are charged and the movement of the analytes are driven by electroosmotic flow (as opposed to hydrodynamic flow). Such a device as claimed in amended claim 1 is not disclosed in the prior art. Therefore, Applicant respectfully requests reconsideration and allowance of pending claims 1, 3 and 5-12.

The specification of the current invention discloses:

In one aspect of the present invention, a membrane was integrated into a microfluidic device for the purpose of concentrating analytes... Through the application of an electric field across the channel, charged analytes were concentrated in front of the membrane, and a concentrated analyte band was ejected from the channel by reversing the polarity of the electric field. In one aspect of the present invention, concentration factors up to 300-fold was measured. In one aspect of the present invention, a plurality of analytes can be concentrated in front of the same membrane without adjustments, provided that they are all anionic or cationic. **In one aspect of the present invention, in the presence of an electric field a charge trapping effect was observed; small molecules can be concentrated in front of membranes with pore sizes which are orders of magnitude above the molecular weight cut-offs for hydrodynamically driven systems...** (Page 10, Lines 3-4, 6-14)(Emphasis added).

For electrically driven concentration, analyte retention in front of the membrane appears to occur primarily by a charge trapping mechanism. In the presence of such a charge trapping mechanism, relatively large pores can be used to concentrate the small molecules, making the system more robust... (Page 10, Lines 17-20)(Emphasis added).

Studies of analyte concentration were performed using fluorescein and R6G in 50mM borate buffer at a pH of 8. At this pH, fluorescein is negatively charged while R6G is positively charged, making the pair of dyes useful for investigation of the effects of analyte charge on retention by the membrane. The results indicate that the analyte charge plays a pivotal role in the retention of analytes. Fluorescein was retained by both the 10-nm and 50-nm pore membranes while the R6G was not retained by either. Membranes with both 10-nm and 50-nm pore sizes were used. **An important observation is that fluorescein (330 g/mole) was concentrated in front of both the 10-nm and the 50-nm pore membranes although these pore diameters are very large with respect to the size of the molecules. Typically, these ultrafiltration membranes are used for separations driven by hydrodynamic flow through the membrane, and the molecular weight cut-offs for hydrodynamic flow are about 1×10^5 for the 10-nm pore membrane and 8×10^5 for the 50-nm pore membrane. Retention and concentration of fluorescein under these conditions demonstrates that the concentration of the dye in front of the membrane and its mass transport across the membrane in electrically driven systems are governed by different mechanisms than those that govern transport in a hydrodynamically driven system. As previously stated, the charge of the molecule plays a dominate role in the concentration as fluorescein could be concentrated using both 10-nm and 50-nm pores, while R6G could not be concentrated by either membrane.** (Page 37, Line 16-Page 38, Line 5)(Emphasis added).

As the above-identified passages clearly disclose, one benefit of the present invention is the ability to concentrate a charged analyte in front of a membrane wherein the pores of the membrane are larger than the pores of the analyte. As disclosed, such a device is possible because of the use of charged analytes in an electrically driven system. With this Response, independent claim 1 has been amended to require a device capable of concentrating analytes in front of a membrane wherein the pores of the membrane are larger than the diameter of the analyte.

To the contrary, the Nelson et al. patent merely discloses a microfluidic device having a membrane (or equivalent structure) capable of blocking or binding to a desired analyte (irrespective of whether the analyte is charged or uncharged) and removing unwanted impurities. The Nelson et al. patent does not disclose or suggest the ability to concentrate a charged analyte in front of a membrane wherein the pore size of the membrane is larger than the diameter of the

analyte. In short, the Nelson et al. patent merely discloses a typical membrane or other device capable of blocking or binding to an analyte, removing a waste stream and then adding a new buffer in order to obtain a desired concentration of analyte.

More specifically, the Nelson et al. patent discloses:

Integrated electrophoretic microdevices comprising at least an enrichment channel and a main electrophoretic flowpath, as well as methods for their use in electrophoretic applications, are provided. **The enrichment channel serves to enrich a particular fraction of a liquid sample for subsequent movement through the main electrophoretic flowpath.** In the subject devices, the enrichment channel and electrophoretic flowpath are positioned such that waste fluid from the enrichment channel does not flow through the main electrophoretic flowpath, but instead flows through a discharge outlet. The subject devices find use in a variety of electrophoretic applications, where entities are moved through a medium in response to an applied electric field. The subject devices can be particularly useful in high throughput screening, for genomics and pharmaceutical applications such as gene discovery, drug discovery and development, and clinical development; for point-of-care in vitro diagnostics; for molecular genetic analysis and nucleic acid diagnostics; for cell separations including cell isolation and capture; and for bioresearch generally. (the Nelson et al. reference; Col. 2, Lines 48-67)(Emphasis added).

Alternatively, or in addition to solid phase materials such as coated particles or other insoluble matrices as the enrichment means, one may employ a coated and/or impregnated membrane which provides for selective retention of the analyte comprising fraction of the sample while allowing the remainder of the sample to flow through the membrane and out of the enrichment means through the waste outlet. A variety of hydrophilic, hydrophobic and ion-exchange membranes have been developed for use in solid phase extraction which may find use in the subject invention. See, for example, Tomlinson et al., "Novel Modifications and Clinical Applications of Preconcentration-Capillary Electrophoresis-Mass Spectrometry," J. Cap. Elect. (1995) 2: 97-104; and Tomlinson et al., "Improved On-line Membrane Preconcentration-Capillary Electrophoresis (mPC-CE)," J. High Res. Chromatogr. (1995) 18:381-3. (the Nelson et al. reference; Col. 6, Lines 30-45)(Emphasis added).

In FIG. 11 there is shown a flow diagram of a device 70, in which there is only one fluid inlet into, and one fluid outlet out from, the enrichment channel 72, as sketched in FIG. 5 and described with reference

thereto. During sample injection by way of the syringe interface the fluid inlet 116 serves as a sample inlet and the fluid outlet 118 serves as a waste outlet. **While the fraction of interest is retained by the retention medium in the enrichment channel, the waste fraction flows downstream through the secondary electrophoretic flowpath 73, across the intersection 82 of the secondary electrophoretic flowpath with the main electrophoretic flowpath 71, and into discharge outlet 84, which directs the waste away from the main electrophoretic flowpath 71 toward waste reservoir 78.** During elution, elution buffer is injected by way of the syringe interface; fluid inlet 116 serves as an elution buffer inlet and the fluid outlet 118 serves as an enriched fraction outlet to the secondary electrophoretic channel. The fraction of interest moves into the elution buffer in which it is driven electrokinetically in an electric field produced by applying a voltage across electrodes 79, 81 to the intersection of the secondary electrophoretic channel and the main electrophoretic channel. Once the fraction of interest has reached the intersection, a voltage is applied across electrodes 76, 77 to draw the analyte or analytes in the fraction of interest into and along the main electrophoretic flowpath to the detection zone 99. (the Nelson et al reference; Col. 15, Lines 10-35)(Emphasis added).

As the above-identified passages indicate, the Nelson et al. patent merely discloses a microfluidic apparatus capable of retaining a desired component in an enrichment channel by reversibly blocking or binding the desired component to a solid structure. As the desired component is bound to the solid structure, an unwanted component is allowed to leave the channel through a waste stream. Next, a desired buffer may be added to the enrichment channel so that the desired component disengages from the solid structure and is now able to achieve a desired concentration (by adding a certain amount of the desired buffer).

As such, the Nelson et al. patent does not disclose a microfluidic device wherein a charged analyte is concentrated in front of a membrane wherein the membrane comprises pores having a larger pore diameter than the diameter of the analyte. While the Nelson et al. patent does disclose that a membrane may serve the function of the solid structure, the Nelson et al. patent does not disclose, teach or suggest the use of a membrane concentrating a charged analyte with a membrane having a larger pore diameter larger than the diameter of the analytes. Such a ratio of pore diameter to analyte diameter is contrary to what one skilled in the art would expect (most would expect the pore diameter to be smaller than the analyte diameter). As such, the embodiment of independent claim 1 cannot be seen as an obvious improvement over the Nelson

et al. patent. Therefore, Applicant respectfully requests reconsideration of pending claims 1, 3 and 5-12 in light of the amendment to independent claim 1.

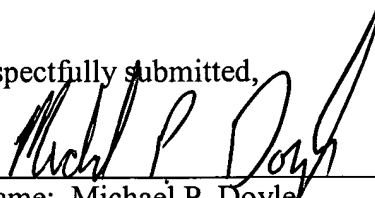
The Wang et al. reference was cited by the December 29th Office Action due to the fact that the Wang et al. reference discloses the presence of an enzyme in the reaction channel. However, the Wang et al. reference does not correct the deficiency of the Nelson et al patent. More specifically, the Wang et al. reference does not disclose a microfluidic device wherein a charged analyte is concentrated in by a membrane wherein the pores of the membrane are larger in diameter than the diameter of the analytes. Therefore, neither the Nelson et al. patent nor the Wang et al. reference, alone or in any combination, disclose, suggest or teach the device of amended independent claim 1. As such, Applicant respectfully requests reconsideration and allowance of pending claims 1, 3 and 5-12.

With this Amendment, Applicant has made an earnest effort to respond to all issues raised in the Office Action of December 29, 2004, and to place all claims presented in condition for allowance. No amendment made was for the purpose of narrowing the scope of any claim, unless Applicant has argued herein that such amendment was made to distinguish over a particular reference or combination of references.

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicant's attorney would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney of record.

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